Sandia National Laboratories
Waste Isolation Pilot Plant
Test Plan TP-02-06

Experimental Study of Microbial Gas Generation Under WIPP-Relevant Humid Conditions

WBS 1.3.5.4.1

Revision 1

Effective Date: 07/25/02

Prepared by:

Y. Wang, 6822
Principal Investigator
WIPP Repository Performance
& Certification Department

APPROVAL PAGE

Prepared by:	*Original signed by Yifeng Wang* Y. Wang, 6822 WIPP Repository Performance & Certification Department	*7/24/02* Date
Technical Reviewer	*Original signed by Nathalie Wall* Nathalie Anne Wall, 6822 WIPP Repository Performance & Certification Department	*7/24/02* Date
QA Reviewer	*Original signed by M. Mitchell* Marty Mitchell, 6820 Carlsbad Programs Group	*7/24/02* Date
Responsible NWMP Manager	*Original signed by F.D. Hansen* F. D. Hansen, 6822 Acting Manager WIPP Repository Performance & Certification Department	*7/24/02* Date

TABLE OF CONTENTS

1.0 DEFINITION OF ACRONYMS

ASTM	American Society for Testing and Materials
BNL	Brookhaven National Laboratory
CCA	Compliance Certification Application
CBFO	Carlsbad Field Office
CRA	Compliance Recertification Application
DAS	Data acquisition system
DOE	Department of Energy
EPA	Environmental Protection Agency
GC-MS	Gas chromatography – mass spectrometer
ICP-OES	Inductively coupled plasma optical emission spectrometer
MGGP	Microbial Gas Generation Program
M&TE	Measuring and test equipment
NIST	National Institute of Standards and Technology
NWMP	Nuclear Waste Management Program
NP	Nuclear Waste Management Program Procedure
PA	Performance assessment
QA	Quality assurance
SNL	Sandia National Laboratories
SOP	Standard Operating Procedure
SP	NWMP Activity/Project Specific Procedure
TOP	Technical Operating Procedure
TP	Test plan
WIPP	Waste Isolation Pilot Plant

2.0 REVISION HISTORY

The original test plan was issued on June 28, 2002. This is the first revision of the test plan. This revision includes a more detailed description of microbe cell counting methods.

3.0 PURPOSE AND WORK SCOPE

The transuranic wastes destined to the Waste Isolation Pilot Plant (WIPP) contain a large quantity of cellulosics, plastics, and rubbers, which may potentially be degraded by microorganisms in the repository over a regulatory time period of 10,000 years via the following sequential reactions (Wang and Brush, 1996):

$$C_6H_{10}O_5 + 4.8H^+ + 4.8NO_3^- \rightarrow 7.4H_2O + 6CO_2 + 2.4N_2 \quad (1)$$

$$C_6H_{10}O_5 + 6H^+ + 3SO_4^{2-} \rightarrow 5H_2O + 6CO_2 + 3H_2S \quad (2)$$

$$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2. \quad (3)$$

Gas generation from biodegradation and metal corrosion would pressurize the WIPP repository, which could lead to an increase in spalling and direct release of radionuclides in the event of human intrusions (Helton et al., 1998). The CO_2 generated from microbial reactions will reduce the pH of WIPP brines and provide additional carbonate ions for actinide complexation, thus resulting in the potential for increased actinide mobility in the repository. To mitigate the effect of microbial gas generation on brine chemistry, an MgO engineered barrier has been designed to sequester CO_2 from aqueous and gaseous phases in the repository (Wang et al., 1997).

The WIPP microbial gas generation program (MGGP) was designed to establish defensible microbial degradation rates for the WIPP Compliance Certification Application (CCA), which was submitted to the Environmental Protection Agency (EPA) in 1996 and approved in 1998. After the CCA submission, the Department of Energy Carlsbad Field Office (DOE/CBFO) decided to continue the MGGP with an objective to increase confidence in the CCA gas generation model and provide more realistic long-term rates for WIPP Compliance Recertification Applications (CRA), because a sensitivity analysis based on the CCA calculations has shown that microbial gas generation rates are among the most sensitive parameters for WIPP long-term performance (Helton et al., 1998). Over the last 10 years, Brookhaven National Laboratory (BNL) has been responsible for the experimental part of the MGGP, while Sandia National Laboratories (SNL) has provided the overall technical guidance and the integration of the experimental data into the performance assessment (PA) models. More background information can be found in two test plans (Brush, 1990; Francis et al., 2001) and various technical reports (Francis and Gillow, 1994, 2000; Wang and Brush, 1996; Francis et al., 1997; Wang et al., 1997; Gillow and Francis, 2001a, 2001b, 2002).

The rate of microbial degradation under humid conditions is an important parameter for the prediction of gas generation for a time period when the repository is not inundated. The existing rate was derived from the BNL "humid" experiments, in which liquid inocula were directly added to cellulosic materials and the humidity of the samples was maintained by placing a glass tube containing G-Seep brine (water activity $a_w = 0.73$, Francis et al., 1997, p. 17) inside each incubation bottle (Francis et al., 1997). The addition of liquid inocula might have artificially increased the water content in cellulosic materials and thus induced a high humid biodegradation rate as observed. A high rate for humid microbial gas generation currently implemented in performance assessment calculations contributes, to a large extent, to the calculated pressure buildup in the repository before a human intrusion and therefore to the spalling and direct release of radionuclides. The spalling release requires the gas pressure to be higher than ~ 80 atm.

In reality, the humid degradation rate is expected to be very low, if not zero. A preliminary natural analog study indicates that the cellulosic materials that have been placed in the Eddy Potash Mine for over 40 years have experienced practically no biodegradation at all under humid conditions (Xu, 2001). For the WIPP, the emplacement of MgO backfill will significantly reduce the water vapor pressure in waste disposal rooms by hydration reaction:

$$MgO + H_2O = Mg(OH)_2. \quad (4)$$

The water activity (a_w) buffered by this reaction is calculated to be $10^{-6.3}$ atm, based on thermodynamic data from Robie et al. (1978). The actual water activity in the repository, which will ultimately be controlled by the relative rates of both brine inflow and MgO hydration, is expected to be much lower than that established by WIPP brines, about 0.7 (Brush, 1990). An abundance of laboratory and field data imply that the minimum water activity required by microorganisms in soils is about 0.6, below which microbial activities are significantly inhibited (Kral and Cousin, 1981; Donnelly et al., 1990; Nizovtseva et al., 1995; Barros et al., 1995; Stark and Firestone, 1995; Cattaneo et al., 1997). Based on all these considerations, it is believed that the humid rate for a WIPP environment should be close to zero and thus the gas generation under humid conditions has been overestimated in the existing PA calculations.

The experiments described in this test plan will focus on the further determination of the rate of microbial gas generation under WIPP-relevant humid conditions by improving the previous experimental procedure. Two major improvements will be made: (1) the extra water introduced into samples by adding liquid inocula will be removed before the start of the incubation experiments; (2) the samples will be maintained at various levels of humidity by using different salt-saturated brines. The results obtained from these experiments will directly support the DOE-proposed modifications to microbial gas generation rates for WIPP CRA.

The work described in this test plan is a natural extension of the existing BNL gas generation studies and will be conducted at SNL-Carlsbad Geochemical Laboratory. Over the past 10 years, BNL has gained a great deal of experience in dealing with WIPP-

related microbial issues. Therefore, the technical assistance from the BNL personnel (A.J. Francis and Jeff Gillow) will be essential for the success of the planned work. The assistance will include the development of appropriate inocula, microbial characterization, experimental design, and data interpretation.

4.0 EXPERIMENTAL PROCESS DESCRIPTION

4.1 Overall Strategy and Process

The following experimental setup and procedure are modified from the BNL microbial gas generation experiments (Francis and Gillow, 1994; Francis et al., 1997).

Experimental setup. The experimental setup for humid incubation will consist of a 160-mL serum bottle and a glass tube placed inside the bottle[1]. The glass tube in each sample will contain an equal volume of salt-saturated brine to maintain a constant humidity in the serum bottle. Cellulosic materials will be placed on the bottom of the bottle. Caution will be taken to avoid any spills of the brine onto the cellulosic materials. The serum bottles will be closed with butyl rubber stoppers. The headspace volume of the samples will be determined by measuring the volume of water needed to fill the whole bottle.

It is anticipated that the WIPP repository will become anoxic shortly after room closure. To simulate the repository condition, all samples need to be incubated under anaerobic conditions. All solutions will be purged with Ar gas before use. The headspace of each sample will be purged with Ar gas before it is tightly sealed. All of the samples will be prepared and sampled inside a glove box purged with Ar gas.

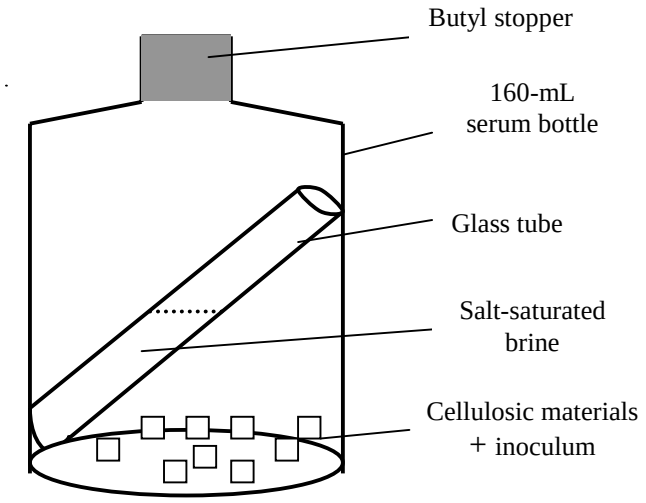

Figure 1. Experimental setup for determining the rate of microbial gas generation under humid conditions.

[1] Other size bottles can be used if 160 mL ones are not available.

Substrates. Cellulosic materials in WIPP wastes consist of ~ 70% of papers and ~ 26% of various woods (Brush, 1990, p. 54). A mixture of four types of papers will be used in the majority of incubation samples: filter paper (Whatman #1™), white paper towel (Fort Howard), brown paper towel, and Kimwipes™ (Kimberly-Clark, lintless tissue wipes). These four types of materials are identical to those used in the BNL experiments. The papers will be shredded into 1-cm-x-1-cm squares. Each type of paper will be weighed (0.25 g), mixed thoroughly, and transferred to serum bottles that have been acid-washed (10% HCl) and sterilized (autoclaved at 120°C for 20 min). A small set of samples will use shredded woods (e.g., saw dust) (1.0 g/sample) rather than papers for a comparison of the biodegradability of papers and woods. In each sample, 1.0 g of cellulosic materials will be mixed with 5.0 g of either crushed WIPP muck pile salt or reagent-grade NaCl.

Inocula. The same inocula will be used as those developed for the BNL experiments. These inocula were prepared from a mixture of a variety of WIPP-relevant samples (Francis et al., 1997). The use of a mixed inoculum instead of a pure culture ensures that a host of microorganisms are present in the experiment and prevents bias toward one potential gas-generating microbial process. Two mL of the mixed inocula will be pipetted onto cellulosic materials in each sample with a calibrated pipette. Each uninoculated sample will instead receive an equal volume of filter-sterilized (0.2 μm) reagent-grade NaCl solution (20% w/v deionized water). The samples will be dried in a desiccator. An appropriate desiccant (e.g., $CaSO_4$) and the duration of the desiccation will be chosen to ensure the viability of microorganisms after the desiccation. The viability of microorganisms will be tested by first rewetting the sample and then checking if microbes can still grow (Gillow, 1996; Gillow, 1998). A small number of samples will be prepared without desiccation of inocula for clarification of the effect of additional water introduced into samples by adding liquid inocula.

Nutrients and electron acceptors. The samples will be prepared with and without added nutrients. The nutrients added will consist of 0.5 mL of solution containing ammonium nitrate (0.1% w/v), potassium phosphate (0.1% w/v), and yeast extract (0.05%w/v). Unamended samples will receive 0.5 mL of filtered, sterilized reagent-grade salt solution (20% w/v). The nutrient solution will be added to samples before the desiccation of inocula. All samples will be prepared in triplicate. See the treatment matrix for sample preparation in Figure 2.

As shown in Reactions 1 and 2, nitrate and sulfate can serve as electron acceptors for microbial degradation. To bound the concentrations of these components in actual wastes, an extra amount of nitrate and sulfate may need to be added to a selected number of nutrient-amended samples.

Humidity control. A constant humidity will be maintained in the samples by glass tubes containing saturated salt solutions (Figure 1). The following salts will be used to buffer the samples at different humidity levels (Lide, 2002; Brush, 1990):

Relative Humidities of Saturated Salt Solutions

Salt	Relative humidity (%) at 25 ^{o}C
WIPP brine	~70
$NaBr.2H_2O$	58
$NaI.2H_2O$	38
$LiCl.H_2O$	11

Ca and Mg salts are excluded because of possible formation of carbonate minerals, which may affect the measurement of CO_2 in the headspace. To minimize the CO_2 uptake by the solution, the pH of the solution will be adjusted to a mildly acid value (e.g., ~ 5). The partition coefficients of gaseous species between the solution and the headspace will be measured and used for the correction for gas dissolution in the solutions.

Incubation. All of the samples will be incubated at ~ 30^{o}C in a temperature-controlled oven over a time period of 3 years.

Inundated samples. A small number of brine-inundated samples will be also prepared. These samples will serve two purposes: (1) one set of these samples will mimic experimental conditions at BNL to provide a consistency check for the data obtained from the work described in this test plan as compared to those obtained at BNL, which is important for deriving a defensible scaling factor for microbial gas generation rates between humid and inundated conditions; (2) by appropriate control of nitrate and sulfate concentrations in synthetic brines, the inundated samples prepared in this work will allow better demonstration of the dependence of the microbial gas generation rate on the identity of the electron acceptor. The treatments of the inundated samples include: (1) 5 g of mixed cellulosic materials, and (2) 100 mL of brine with or without dissolved sulfate. Most of the samples will be amended with nutrients or excess nitrate; some will be unamended for comparison. The amounts of nitrate and sulfate added to the samples will reflect and bound the concentrations of these components in actual wastes. Synthetic G-Seep brine will be used (Brush, 1990):

Composition of G-Seep brine

Major ion	g/L	M
Na^{+}	95.0	4.11
Cl^{-}	181	5.10
Mg^{2+}	15.3	0.63
K^{+}	13.7	0.35
Ca^{2+}	0.32	0.01
SO_4^{2-}	29.1	0.30
HCO_3^{-}	0.73	0.01

All the samples will be prepared under anoxic conditions.

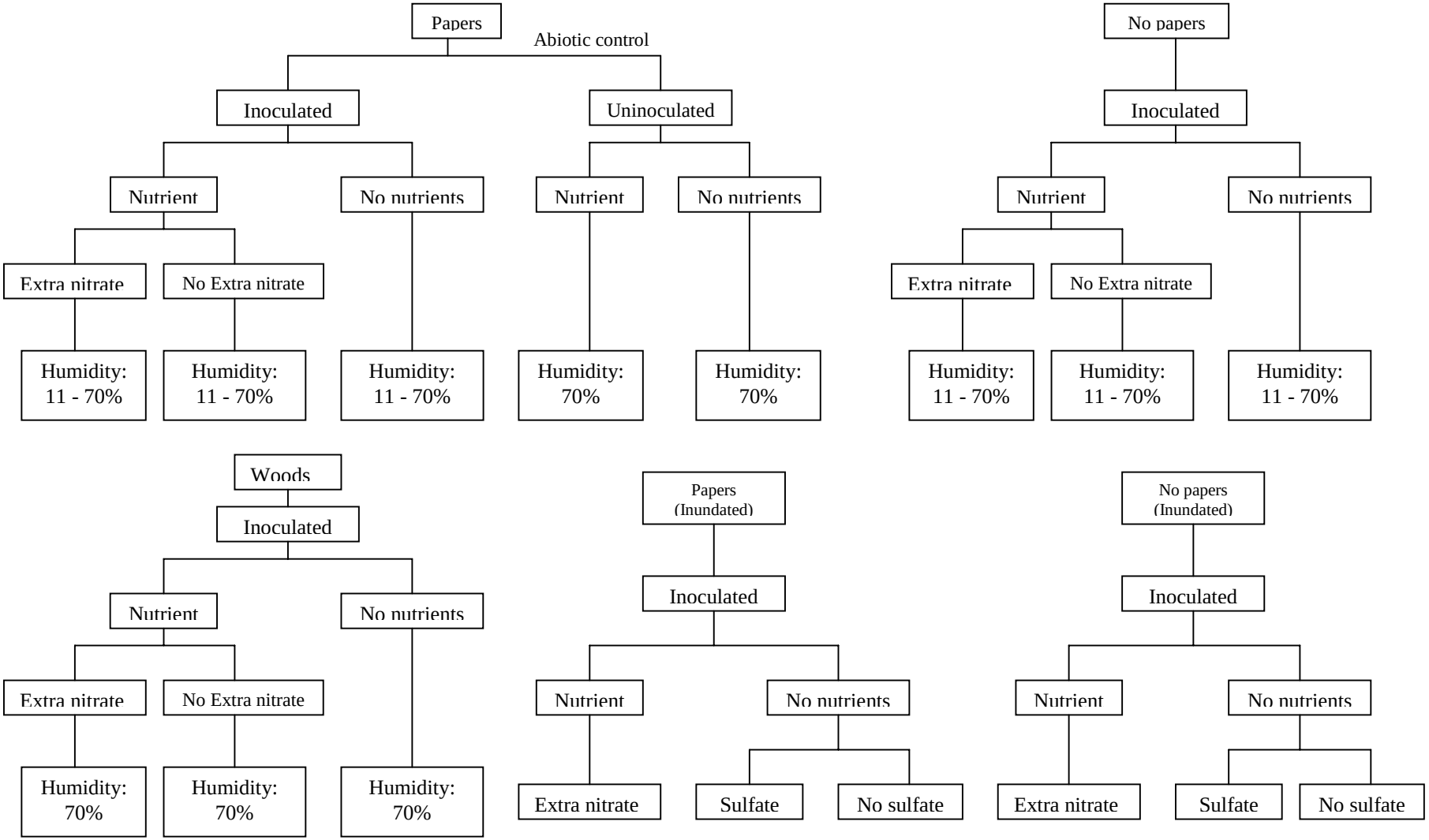

Figure 2. Treatment matrix for samples. Each sample will be prepared in triplicates. At minimum, a total of 105 samples are needed.

Abiotic controls. Samples containing sterilized, reagent-grade NaCl without inoculum will serve as abiotic controls. Cellulosic materials in these samples will be sterilized prior to the experiments. Formalin may also be used for the abiotic controls.

Gas sampling and analysis. The composition of the headspace gas of each sample will be monitored over time. The headspace gas will be sampled at least once a month for the first year and less frequently thereafter depending on the overall gas generation rate. For each sampling, the butyl rubber septum on the serum bottle will be pierced with a sterile, 22-gauge needle (Becton Dickenson) attached to a digital pressure gauge to measure the headspace gas pressure for the calculation of total generated gas. Immediately after this, a gas-tight syringe (Pressure-Lok™, Precision Instrument Corp.) fitted with a stainless-steel side-port needle will be used to take 10 μL of headspace gas to determine the various gases (Ar, CH_4, CO_2, H_2, H_2S, N_2, and O_2) quantitatively by Varian 3900 Gas Chromatography (GC) with a Saturn 2100T Ion Trap Mass Spectrometer (MS). The Rubber stopper will not be replaced after each sampling. It is expected that the gas leakage, if any, from the pierced rubber stopper is negligible, as long as the bottle is not overly pressurized. Ar will be used as an inert gas to check for gas leakage from the serum bottles during the experiments, and its initial concentration will be analyzed immediately after the bottle is closed.

Bacterial counting. Bacterial direct counting will be needed to check the viability of microbes in dried inocula. Microbial cells will be stained with the DNA-specific stain 4'6-diamidino-2-phenylindole (DAPI, Polysciences, Inc.) and will be examined by epi-fluorescence microscopy using Olympus BX60 Polarizing Microscope and UV light source (Kepner and Pratt, 1994; Gillow, 1996, 1998). Alternatively, green-fluorescent nucleic-acid dyes will be used in conjunction with aluminum oxide filters if particulate matter in the dried inoculum complicates microscopic visualization (Weinbauer, 1998). Direct counting may be used as the experiment progresses to detect increases in the microbial population.

Data reduction. Gas generation rates will be determined from total gas volume and individual gas concentrations accumulated with time. The total amount of gas in the headspace determined from the pressure measurement will be compared with that determined from GC-MS to ensure data quality.

4.2 Sample Control

The sample control for the work under this test plan will follow WIPP Nuclear Waste Management Program (NWMP) Procedure NP 13-1. Each sample will be appropriately labeled. Sample preparation, utilization, and final disposition will be documented in scientific notebooks. When samples are not in the possession of individual designated with their custody, they will be stored in a secure area with associated documentation (chain of custody).

4.3 Data Quality Control

Measuring and Test Equipment (M&TE). A calibration program will be implemented for the work described in this test plan in accordance with NP 12-1, "Control of Measuring and Test Equipment." This M&TE calibration program will meet the requirements in NWMP procedure NP 12-1 for: (1) receiving and testing M&TE; (2) technical operating procedures for M&TE; (3) the traceability of standards to nationally recognized standards such as those from the National Institute of Standards and Technology; (4) maintaining calibration records. In addition, NP 13-1 and SP 13-1 identify requirements and appropriate forms for documenting and tracking sample possession.

Data Acquisition Plan. Data collection procedures are specific to individual instruments. For details of the data acquisition for a particular instrument, see the specific procedures (SP) or users manual for that instrument. Any data acquired by a data acquisition system (DAS) will be attached directly to the scientific notebook or compiled in separate loose-leaf binders with identifying labels to allow cross reference to the appropriate Scientific Notebook. If the instrument allows data to be recorded electronically, copies of the data disks will be submitted to the NWMP Records Center according to NWMP procedure NP 17-1, "Records." For instruments that do not have direct data printout, the instrument readings will be recorded directly into the scientific notebook. Current versions of the DAS software will be included in the SNL WIPP Baseline Software List, as appropriate.

Quality control of the scientific notebooks will be established by procedures described in NWMP procedure NP 20-2, "Scientific Notebooks." Methods for justification, evaluation, approval, and documentation of deviation from test standards and establishment of specially prepared test procedures will be documented in the Scientific Notebooks. Procedures including use of replicates, spikes, split samples, control charts, blanks and reagent controls will be determined during the development of experimental techniques.

The numerical data will be transferred from data printouts and scientific notebooks to Microsoft Excel (Office 97 version or later) spreadsheets. Data transfer and reduction will be performed in such a way to ensure that data transfer is accurate, that no information is lost in the transfer, and that the input is completely recoverable. Data transfer and reduction will be controlled to permit independent reproducibility by other qualified individuals. A copy of each spreadsheet will be taped into the scientific notebook, and a second person will compare the data recorded in the notebook and that on the spreadsheet to verify that no transcription errors have occurred during technical and/or QA review of the notebook. This verification will be documented in the notebook when signed by the reviewer.

Data Identification and Use. The details about the data to be obtained and the use of these data can be found in Section 4.1. All calculations performed as part of the activities of TP 02-06 will be documented in a scientific notebook. The notebook will be technically reviewed periodically by a second person, who will note concurrence by co-signing the examined material. If a discrepancy is found, that discrepancy and its

resolution will be documented in the notebook. In addition, there will be periodic quality assurance reviews of the notebook to ensure that the requirements of NWMP procedure NP 20-2, "Scientific Notebooks" are addressed.

4.4 Equipment

A variety of measuring and analytical equipment will be used for the work described in this test plan. A complete equipment list, including serial numbers, will be maintained in the scientific notebook. For a newly purchased instrument, if the operating procedure has not yet been developed or written, scientific notebooks will be used to record all laboratory work activities.

Weighing Equipment. Several balances are present in the facility and may be used for this project. These include a Mettler AT-261 five-decimal-place electronic balance, an ANC three-decimal-place balance, and top-loading balances and scales with maximum ranges of 2 to 30 kilograms. Balance calibration checks will be performed routinely using the following NIST-traceable weight sets, which, in turn, are calibrated by the SNL Calibration Laboratory every three years:

- *Troemner Calibration weight set*, ASTM Class 1, Serial number 22803, 1 mg – 100 g, calibration expires 12/16/02.
- *Troemner Calibration weight*, NIST Class 1, Serial number 42795, 100 g, calibration expires 11/19/02.
- *Troemner Calibration weight*, NIST Class 1, Serial number 42797, 100 g, calibration expires 11/19/02.
- *Troemner Calibration weight*, NIST Class 1, Serial number 42799, 100 g, calibration expires 11/19/01.
- *Troemner Calibration weight*, NIST Class 1, Serial number 42800, 100 g, calibration expires 11/19/01.
- *Troemner Calibration weight*, ASTM Class 1, Serial number 47824, 200 g, calibration expires 11/19/02.
- *Troemner Calibration weight*, ASTM Class 1, Serial number 55335, 1000 g, calibration expires 11/19/02.
- *Troemner Calibration weight*, ASTM Class 2, Serial number I-12, 10 kg, calibration expires 12/17/02.

Additional calibrated NIST-traceable weight sets that would be purchased at a later date may also be used. Balance accuracy and precision will be checked daily or prior to use (whichever is less frequent), using the calibration weight sets listed above. Calibration checks will be recorded in the scientific notebook.

Liquid Measuring Equipment. Standard Laboratory Class A glassware (pipettes, volumetric flasks, etc.) will be used at all times. In addition, several adjustable Eppendorf pipettes are available for use in the laboratory. The calibration of pipettes will

be checked routinely against a calibrated balance, and will be recorded in the scientific notebook.

Gas Analysis Equipment. Varian 3900 Gas Chromatography (GC) with Saturn 2100T Ion Trap Mass Spectrometer (MS) will be used to analyze headspace gas compositions. This is a newly purchased equipment, and the operating procedure needs to be developed.

Gas Sampling Equipment. 22-gauge needles (Becton Dickenson), a digital pressure gauge, and a gas-tight syringe (Pressure-Lok™, Precision Instrument Corp.) fitted with a stainless-steel side-port needle.

Gas Calibration Standards. Appropriate concentrations of analyte gases CO_2, CH_4, H_2, H_2S, N_2, and O_2 will be obtained from commercial sources. Primary calibration standards, specifically for CO_2, will be certified traceable to NIST.

Atmosphere Control Equipment. A glove box will be used for sample preparation and sampling.

Temperature Control Equipment. All samples will be incubated in a temperature-controlled incubator. Temperature will be monitored using instruments that have been calibrated with temperature standards traceable to NIST.

Other Analytical Equipment.

- *pH Meters and Autotitrators* – Solution pH may be measured using pH meters and/or autotitrators. A Mettler Model MA235 pH/Ion Analyzer and a Mettler Model DL25 Autotitrator will be used for this purpose. The range for all pH meters is 0.00 to 14.00. Electrodes will be calibrated before each use or daily (whichever is less frequent) with pH 4, 7, and 10 buffers manufactured by Fisher Scientific with unique lot numbers and expiration dates; traceable to the National Institute of Standards and Technology (NIST). The accuracy of the buffers is ±0.01 pH units; buffer values will be adjusted for laboratory temperatures as per buffer instruction sheets if necessary. Calibration checks will be recorded in the scientific notebook. Measuring pH in concentrated brines is difficult, and a procedure will be developed to calibrate pH meters.
- *Equipment for Chemical Analysis* – Three instruments may be used for chemical analyses. The first is a Perkin Elmer Optima 3300 DV inductively coupled plasma optical emission spectrometer (ICP); the second is a Cary 300 UV-visible spectrophotometer; and the third, is a UIC, Inc. carbon analyzer, consisting of an acidification module, a furnace module, and a CO_2 coulometer. These instruments will be user-calibrated each time they are used and documented in the scientific notebook.
- *Equipment for microbe counting/sizing* – An Olympus BX60 polarizing microscope will be used for observation of microbe preparations and for counting/sizing. The counting grid will be standardized with a Wild stage micrometer, serial # 2660 (or

equivalent), calibrated every five years by Klarman Rulings (NIST-traceable). Sizing accuracy checked using Bangs Laboratories NIST-traceable uniform microspheres (0.538 and 1.900 µm).

NMWP Activity/Project Specific Procedures (SPs) will be written for these instruments as necessary. Until that time, detailed procedure descriptions will be documented in laboratory notebooks.

5.0 TRAINING

All personnel involved in the experiments described in this test plan will be trained and qualified for their assigned work. This requirement will be implemented through NWMP procedure NP 2-1, "Qualification and Training." Evidence of training to assigned NPs, SPs, TOPs, TP 00-07, ES&H procedures, and any other required training will be documented through Form NP 2-1-1, "Qualification and Training." Annual Refresher QA training will ensure on-site personnel are trained to the NWMP QA Program. Specifically, the following Nuclear Waste Management Program Procedures (NPs) and Activity/Project Specific Procedures (SPs) are applicable:

- SOP-C001, "Standard Operating Procedure for Activities in the SNL/Carlsbad Laboratory Facility,"
- SP 12-1, "Use of Laboratory Balances and Scales,"
- SP 12-2, "Use and Maintenance of the UIC, Inc. Model CM5014 CO2 Coulometer, CM5130 Acidification Module and CM5120 Furnace Apparatus,"
- SP 13-1, "Chain of Custody,"
- NP 2-1, "Qualification and Training,"
- NP 6-1, "Document Review Process,"
- NP 12-1, "Control Of Measuring And Test Equipment,"
- NP 13-1, "Sample Control,"
- NP 17-1, "Records,"
- NP 20-2, "Scientific Notebooks."

In addition, SPs will be written for use of the GC-MS, ICP-OES, UV-Vis Spectrophotometer. Sample preparation procedures, which may vary from sample to sample as work scope evolves, will be detailed in scientific notebooks in accordance with NWMP procedure NP 20-2.

6.0 HEALTH AND SAFETY

All of the health and safety requirements relevant to the work described in this test plan and the procedures that will be used to satisfy these requirements are described in ES&H standard operating procedures. SOP-C001 describes the non-radiological hazards associated with these experiments and describes the procedures to deal with those hazards, including all the training requirements for personnel involved in conducting the

experiments. Additional SPs may be mandated by SNL ES&H requirements and their issuance will not require revision of this test plan.

7.0 PERMITTING/LICENSING

There are no special licenses or permit requirements for the work described in this Test Plan.

8.0 REFERENCES

Barros N., I. Gomez-Orellana, S. Feijoo, and R. Balsa. 1995. "The effect of soil moisture on soil microbial activity studied by microcalorimetry,." *Thermochimica Acta*. Vol. 249, pp. 161-168.

Brush, L.H. 1990. *Test Plan For Laboratory and Modeling Studies of Repository and Radionuclide Chemistry for the Waste Isolation Pilot Plant*. SAND90-0266. Albuquerque, NM: Sandia National Laboratories.

Cattaneo, M.V., C. Mason, and C.W. Greer. 1997. "The influence of moisture on microbial transport, survival and 2,4-D biodegradation with generally marked *Burkholderia cepacia* in unsaturated soil columns," *Biodegradation*. Vol. 8, pp. 87-96.

Donnelly, P.K., J.A Entry, D.L. Crawford, and K. Cromack Jr. 1990. "Cellulose and lignin degradation in forest soils: response to moisture, temperature, and acidity," *Microbial Ecology*. Vol. 20, pp. 289-295.

Francis, A.J., and J.B. Gillow. 1994. *Effects of Microbial Processes on Gas Generation under Expected WIPP Repository Conditions: Progress Report through 1992*. SAND93-7036. Albuquerque, NM: Sandia National Laboratories.

Francis, A.J., and J.B. Gillow. 2000. "Progress report: microbial gas generation program." Unpublished memo to Y. Wang, January 6, 2000. Carlsbad, NM: Sandia National Laboratories.

Francis, A.J., J.B Gillow, and M.R. Giles. 1997. *Microbial Gas Generation under Expected Waste Isolation Pilot Plant Repository Conditions*. SAND96-2582. Albuquerque, NM: Sandia National Laboratories.

Francis, A.J., J.B Gillow, and Y. Wang. 2001. "Re-Evaluation of Microbial Gas Generation under Expected Waste Isolation Pilot Plant Conditions." TP-99-01, Rev. 1. Carlsbad, NM: Sandia National Laboratories.

Gillow, J.B. 1996. "BNL-EPI, The Direct Count Method for Enumerating Bacteria," Technical Operating Procedure, Upton, NY: Brookhaven National Laboratory.

Gillow, J.B. 1998. "BNL-UV/Vis-1, Analysis of Optical Density of Biomass by spectrophotometry." Technical Operating Procedure, Upton, NY: Brookhaven National Laboratory.

Gillow, J.B., and A.J. Francis. 2001a. "Re-evaluation of microbial gas generation under expected Waste Isolation Pilot Plant conditions." Unpublished report, January 31, 2001. Carlsbad, NM: Sandia National Laboratories. Pp. 19-46.

Gillow, J.B. and A.J. Francis. 2001b. "Re-evaluation of microbial gas generation under expected Waste Isolation Pilot Plant conditions." Unpublished report, July 31, 2001. Carlsbad, NM: Sandia National Laboratories. Pp. 3.1 to 3.21

Gillow, J.B., and A.J. Francis. 2002. "Re-evaluation of microbial gas generation under expected Waste Isolation Pilot Plant conditions." Unpublished report, January 31, 2002. Carlsbad, NM: Sandia National Laboratories. Pp. 2.1-1 to 2.1-25.

Helton J.C., J.E. Bean, J.W. Berglund, F.J. Davis, K. Economy, J.W. Garner, J.D. Johnson, R.J. Mackinnon, J. Miller, D.G. O'Brien, J.L. Ramsey, J.D. Schreiber, A. Shinta, L.N. Smith, D.M. Stoelzel, C.T. Stockman, and P. Vaughn. 1998. *Uncertainty and Sensitivity Analysis Results Obtained in the 1996 Performance Assessment for the Waste Isolation Pilot Plant*. SAND98-0365. Albuquerque, NM: Sandia National Laboratories.

Kepner, R.L., Jr, Pratt, J.R. 1994. "Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present." Microbiological Reviews. Vol. 58, pp. 603-615.

Kral, D.M., and M.K. Cousin. 1981. "Water Potential Relations in Soil Microbiology." SSSA Special Publication no. 9. Soil Science Society of America.

Lide, D.R. 2002. *Handbook of Chemistry and Physics*. Boca Raton, FL: CRC Press.

Nizovtseva, D.V., A.M. Semenov, and N.S. Panikov. 1995 "Influence of humidity on microbial cellulose activity in peat from a raised bog," *Microbiology*. Vol. 64, pp. 701-705.

Robie, R.A., B.S. Hemingway, and J.R. Fisher. 1978. *Thermodynamic Properties of Minerals and Related Substances at 298.15 K and 1 Bar (10^5 Pascals) Pressure and at Higher Temperatures*. U.S. Ge0ological Survey Bulletin no. 1452.

Stark, J.M., and M.K. Firestone. 1995. "Mechanism for soil moisture effects on activity of nitrifying bacteria," *Applied and Environmental Microbiology*. Vol. 61, pp. 218-221.

Wang, Y., and L.H. Brush. 1996. "Estimates of gas-generation parameters for the long-term WIPP performance assessment. Unpublished memo to M.S. Tierney, January 26, 1996. WPO #30819. Albuquerque, MM: Sandia National Laboratories.

Wang, Y., L.H. Brush, and R.V. Bynum. 1997. "Use of MgO to mitigate the effect of microbial CO_2 production in the Waste Isolation Pilot Plant." *WM'97*, March 2-6, 1997, Tucson, Arizona.

Weinbauer, M. G., Beckmann, C., Hofle, M. G. 1998. "Utility of gree fluorescent nucleic acid dyes and aluminum oxide membrane filters for rapid epifluorescence enumeration of soil and sediment bacteria." Applied and Environmental Microbiology. Vol. 64, pp. 5000-5003.

Xu, H. 2001. "Microstructure investigation of cellulosic materials from the Eddy Potash Mine." Unpublished report, January 31, 2001. Carlsbad, NM: Sandia National Laboratories. Pp. 47-54.